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Thesis

THE TRYPANOSOMES OF RODENTS

by

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Master of Arts

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INTRODUCTION

Delving into the literature on rodent trypanosomes especially the rat trypanosome, Trypanosoma lewisi, has proven to be an interesting and sometimes fascinating study. Although much information has been accumulated on the subject it is not rodent trypanosomiasis per se that has been the motivating force of this thesis. To my mind the studies on the rodent disease are valuable only as a convenient source of data which when properly evaluated and applied may furnish further knowledge of the human infections.

The purpose of the thesis, therefore, is to point out the significant advances that have been made since scientific interest first was focused on the rodent trypanosomes. T. lewisi because of its accessibility has been the subject of much more extensive investigation than other rodent trypanosomes and has therefore been utilized as the representative member of the rodent parasites. A section, however, has been devoted to the trypanosomes of numerous other rodents.

I. HISTORICAL

Credit for the discovery of the trypanosome goes to Valentin of Berne who in 1841 observed the parasite in the blood of the trout.[28] Closely following Valentin's discovery were papers by Gluge of Brussels, Mayer of Bonn and Gruby of Paris on frog trypanosomes which were published in 1842 and 1843.[28] It was Gruby who devised the genus name for the new protozoan. From his observations on the frog haemoflagellate he called it "trypanosoma" from the Greek words, "trupanon" (wimble or auger), and "soma" (body).[28] Until the research of Lewis in 1878 the genus remained relatively unexplored except for the work of Gros who in 1845 discovered trypanosomes in the blood of the field mouse and mole and Chaussat who found them in the blood of the black rat (1850), but mistook them for a nematode embryo.[28]

Lewis'[29] work on the blood of rats in India precipitated a new interest in mammalian trypanosomes. Carrying out routine blood examinations on various animals while searching for a possible reservoir for the spirillum of "Bombay Fever" Lewis in 1877 chanced upon some organisms which he at first considered to be bacterial in nature. "They were pale, translucent beings without any trace of visible structure or granularity." The quotation conveys an impression of curiosity and surprise. Further observations on hanging drop and stained preparations of the blood convinced Lewis of the

protozoan nature of the organism particularly when he saw a flagellum. Twenty-nine percent of the rats examined were positive for the organism. This figure compared to a seventeen percent infection reported by Warren[50] in Boston during the winter months is surprisingly low when the more favorable climate of India is taken into account.

In the light of later experimentation which established the rat flea as the probable intermediate host of the rat trypanosome the observation that "organisms often were found in blood of rats of the same brood" is significant. An uninfected rat would probably become infected by the bite of fleas transmitted to it by an infected rat of the same brood.

His curiosity aroused by the work of Lewis in India, Crookshand[10] published a paper in 1886 which was the first to give a comparatively accurate and scientific description of T. lewisi. Ignorant of the fact that only wild, flea-infested rats harbored the parasite Crookshank examined the blood of white rats with no success. However, twenty-five percent of London sewer rats were found to be infected with organisms resembling those described by Lewis. "The first question...was whether...these...were identical with those described by Lewis in Indian rats." The resulting investigation followed a much more terse and scientific method of attack than Lewis' paper. For example, Crookshank noted that the flagellum is continuous with the distal edge

of the undulating membrane. Using "saturated tincture of methyl violet and fuchsin" he was able to make valuable observations by varying the intensity of the stain.

Since the trypanosome of Surra had been discovered by Evans in 1880 Crookshank evidently had little conception of host specificity when he suggested that it and the rat haemoflagellate were morphologically identical. His views are probably symptomatic of scientific thought at the time when he evinced puzzlement at the presence of the organism both in sick horses and other equines and normal, healthy-appearing rats. Strangely enough, Crookshank regarded the surra parasite as the result rather than the cause of the disease. With the latter conception it is not surprising that he was confused by the presence of a similar organism in a healthy animal. The role of the tsetse fly in the transmission of trypanosomiasis of higher mammals being unknown at the time, he suggested that water might be the vehicle for the surra parasite. With a true scientific attitude, however, Crookshank was aware of conflicting observations and the failings of knowledge in his time.

Sent to Zululand to investigate Nagana, a cattle disease, Bruce[7] in 1897 discovered the trypanosome which now bears his name. "It was the first time the writer had seen a trypanosome." Of greatest significance was his demonstration that wild game (buffaloes and antelopes) constituted a reservoir, the tsetse fly being the vector of

the Nagana parasite.

A few years later (1901) a European suffering with pyrexia and splenomegaly came to the attention of Dr. R. M. Forde[17] in Gambia. Diagnosing the case as malaria he administered quinine but the patient showed no improvement. It was after the examination of blood smears that, "I found no malarial parasite... but found...small, worm-like....bodies ...which I prematurely pronounced a species of filaria." Invalided to Europe the patient was examined by Dr. Dutton of the Liverpool School of Tropical Medicine who recognized the parasite to be a species of trypanosome. The discovery of a human trypanosome by Forde and Dutton provided an additional stimulus to tropical medicine research in the early twentieth century. It must be emphasized that Forde was the first to see the parasite, but Dutton receives the credit for the identification of the new human parasite.

Working in Uganda in 1903 Castellani[6] discovered trypanosomes in the cerebrospinal fluid and blood of patients suffering with sleeping sickness. Member of a Sleeping Sickness Commission sent to replace a similar commission headed by Castellani Dr. Bruce[6] was told of the discovery and encouraged him to stay on and finish his phase of the work. The result was seventy percent of the cases examined were positive for trypanosomes. Castellani's discovery definitely demonstrated the relation of trypanosomes to recognized cases of sleeping sickness. Put on the right

track by Castellani, Bruce, Nabarro, and Greig produced evidence that the trypanosome is the etiological agent of sleeping sickness, the tsetse fly being the vector of the human infection. It should be stressed that the organisms (T. gambiense) discovered by Forde and Castellani are identical but it was the latter who found them in patients with definite clinical symptoms of sleeping sickness.

Further investigations proved trypanosomes to the causative agents of other diseases but they did not come within the scope of this thesis and will, therefore, not be considered.

II. CLASSIFICATION

The Family Trypanosomidae

In his paper on the rat trypanosome, Crookshank[10] in 1886 suggested that the microorganisms now called trypanosomes be placed in the genus, Trichomonas, subgenus, Trichomonas sanguinis. Since he was not aware of host specificity he further suggested that both the rat and the hamster parasites be called Trichomonas lewisi. This confusion of trypanosomes and trichomonads was subsequently cleared, all blood-inhabiting, parasitic protozoa possessing undulating membranes being placed in one genus, Trypanosoma. The genus is of course placed under the class Mastigophora, while all flagellates closely related to the trypanosome are embraced by the family, Trypanosomidae, on the basis of mutual morphological attributes.[54] All organisms considered to be members of the family possess a nucleus and a flagellum arising from a kinetoplast which, in turn, is composed of a posterior, more deeply-staining parabasal body and an anterior blepharoplast in which the flagellum arises.

Generally speaking, trypanosomes undergo four developmental stages: leishmania, leptomonas, crithidia, and finally the true, trypanosome stage. Curiously enough, each of the first three stages exists as a separate genus which indicates the possible evolutionary path in the development of the true trypanosome type. The transformation from the primitive,

leishmania form to the adult trypanosome and the inter-relationship of each stage can be best grasped by the study of representative diagrams (See fig. 1). The maturation of the trypanosome and hence, the most likely pattern of evolution consists in the development of a flagellum arising from a kinetoplast lying anterior to the nucleus simultaneously with the formation of an axoneme traveling along the distal end of a freely extending cytoplasmic fold, the undulating membrane, and the gradual posterior migration of the kinetoplast past the nucleus to the caudal end of the organism with a corresponding increase in the length of the body, axoneme, and undulating membrane.

Evolution of the Vertebrate Haemoflagellate

The role of the rat flea as the vector of rat trypanosomiasis has been demonstrated by the exhaustive research of Minchin and Thomson[33] who investigated the immature forms found in the rat flea, Ceratophyllus fasciatus. In the intermediate, insect host the immature forms display a wide variation of morphology, the gamut from leishmania to trypanosome forms being represented. The fact that the trypanosome undergoes a cycle of development in an intermediate, insect host raises the question of ancestry. Discussing this problem, Wenyon[54] states that it has not yet been agreed whether trypanosomes were blood parasites of vertebrates which by passing into the alimentary canal of

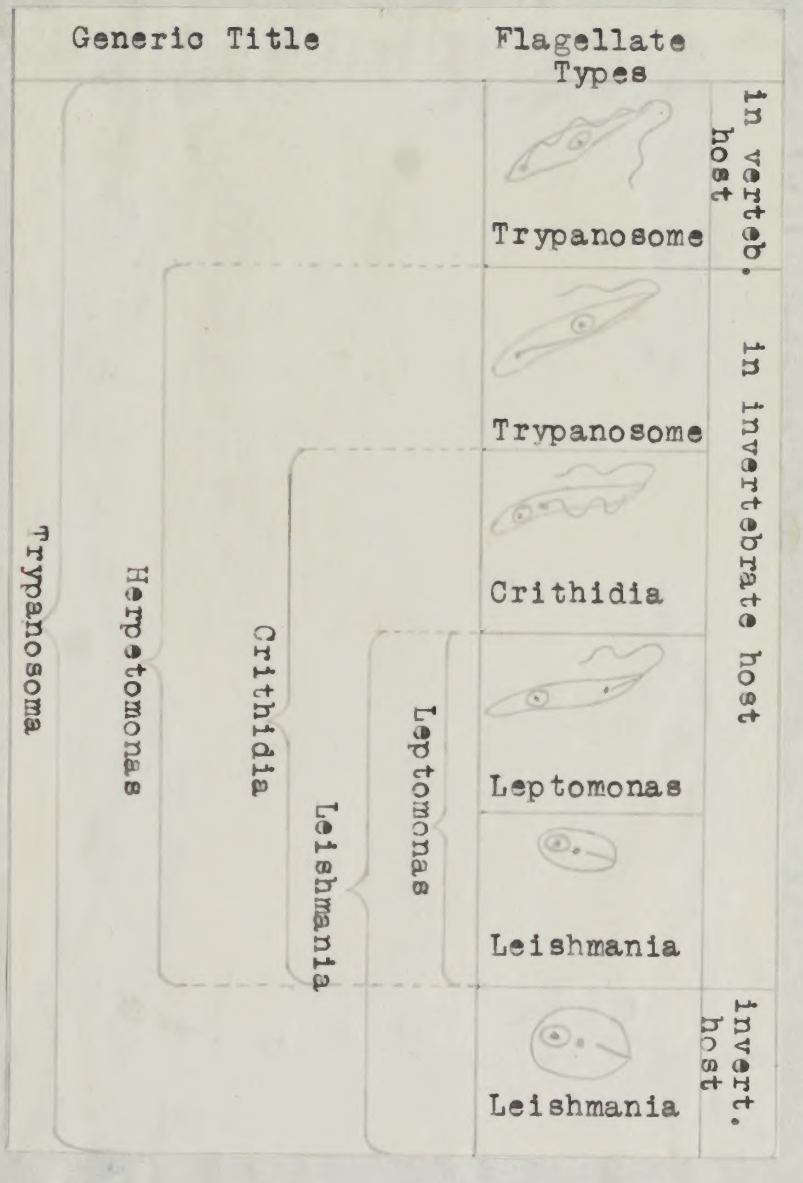

Fig. 1- Diagram of the classification of the trypanosomes and allied flagellates. (After Wenyon)

blood-sucking insects became adapted to existence therein or whether the organism originally was specific to an insect host and adjusted itself to a blood medium when the insect became a blood-sucker on vertebrates or deposited its excreta on the host which licked up the metacyclic trypanosomes. Wenyon holds the latter hypothesis to be the more acceptable. Brumpt's comment on the transmission of rodent trypanosomes is interesting:

> Le fait que ces trypanosomes...sont transmis exclusivement par les déjections est encore en faveur de cette thèse; il démontre que la maladie du vertebré est un phénomène tout à fait accidentel et que ces trypanosomes sont encore peu adaptés.

In other words, the cycle within the insect host being much more complex than that of the vertebrate host, it would seem likely that trypanosomes are primarily insect parasites.

III. BIOLOGY OF THE TRYPANOSOME

Morphology and Cytology

Generally speaking, the adult trypanosome is an elongate, spindle-shaped microorganism with a curved, flexible body. T. lewisi measures 13 to 25 and 2 to 4 micra in length and width respectively, the flagellum being from 7 to 15 micra. Arising caudad at the kinetoplast is the axoneme which forms a border for the distal edge of the undulating membrane and continues craniad past the nucleus out into the free flagellum. The undulating membrance being longer than the axoneme it is thrown into folds.

According to Wenyon[54] the cytoplasm is clear and alveolar in structure and may be vacuolated. Laveran and Mesnil[28] mention vacuolated forms as occurring only in organisms isolated from body fluids other than blood such as edema or cerebrospinal fluid. However, Warren[50] noticed vacuolation in trypanosomes observed in smears made from the heart's blood of rats kept choloroformed for four days. Vacuolation, therefore seems to be associated with any deviation of and from the normal blood medium. Bordered by a periplast, the cytoplasm exhibits no endo-ectoplasmic differentiation and may possibly be traversed by longitudinal, contractile fibers called myonemes. Granules with a great affinity for certain chromatin stains have been observed within the cytoplasm and take a red or purple color with Romanowsky staining.

Regarding T. lewisi, Wenyon[54] discusses a deeply-staining granule surrounded by a clear halo adjacent to the nucleus and occurring in both rod-shaped and double forms; its function has not yet been elucidated. Approximately 11 micra (MacNeal) anterior to the blepharoplast of T. lewisi lies the nucleus, containing a karyosome staining deeply with chromatin stains, which is bordered by a nuclear membrane. The presence of several deeply-staining structures rather than just one karyosome has been noted. MacNeal[31] has seen the chromatin assume a 12-chromosome-configuration.

At the posterior end of the parasite lies a quasi-compound structure from which the axoneme takes root and continues on to the lateral border of the undulating membrane. The kinetoplast, as it is called, consists of the anterior blepharoplast and posterior parabasal body. MacNeal[31] refers to the former as a "smaller chromatin body" having a firm connection with the flagellum since they remain together when the rest of the cell has disintegrated. However, some of his specimens displayed an achromatic space between blepharoplast and flagellum. Since the first indications of division are seen in the kinetoplast numerous speculations have been raised as to the possibility of its nuclear origin or nature. The blepharoplast is the first structure to divide and is therefore the element considered to be of nuclear origin. Rabinowitsch and Kempner[41] thought it to be a nucleolus because of its role in fission. Some authors

assigned the term, "micronucleus" to the blepharoplast, but Laveran and Mesnil[28] believe that the term should be reserved for ciliates since the micronucleus of ciliates is not physiologically analagous to the blepharoplast. Now that the relationship between the latter and the flagellum has been established the conceptions of micronucleus and nucleolus have been withdrawn. The misronucleus of ciliates takes part in a specialized form of mitotic division in conjunction with binary fission. In conjugation an exchange of micronuclear material occurs between the conjugants and has been interpreted to be a sexual or rejuvenatory process, while in endomixis the micronucleus is concerned with a restorative, reproductive mechanism.[36] Sufficient evidence cannot be found in the literature to establish an anology between micronucleus and the blepharoplast or "centrosome" as it is called by Francis[18] who does not advance any theory as to the nuclear nature of the blepharoplast. In his research on avian trypanosomiasis Danilewsky[13] was among the first to establish the intimate relationship between the flagellum and the undulating membrane.

Taking root in the blepharoplast the flagellum forms the lateral border of the undulating membrane and loses its connection with the latter at the anterior end where it projects as the free flagellum. Studies indicate that the flagellum consists of a cytoplasmic sheath enclosing an axial filament, the axoneme. Attached to the convex side

of the body the undulating membrane is believed to be of the same cytoplasmic composition as the body proper.[54] Since the distal edge is longer than the exoneme it is conveniently thrown into folds which probably enhance its powers of locomotion. Considered to be of the same protoplasmic composition as the body it would naturally follow that myonemes might also be responsible for movement of the membrane, but undulation is believed to be caused by axoneme contractions.[54]

A highly motile organism the trypanosome is propelled by the alternate right to left lashings of the flagellum, the undulating motion of the membrane and body movements caused by myoneme contractions. Reporting his observations on the motion of T. lewisi Francis[18] describes it as rotating screw-like on the longitudinal axis of the body and darting among the blood cells flagellum-first without entering or engulfing them.

Reproduction

It is convenient to consider reproduction of the trypanosome in two phases; the simple multiplication of the true trypanosome type within the circulatory system of the host after a developmental cycle in the secondary host or insect vector has been completed. In the insect the parasite experiences various metamorphoses which range morphologically from the leishmania to the crithidia and true trypanosome forms. Although both crithidial and adult trypanosome types

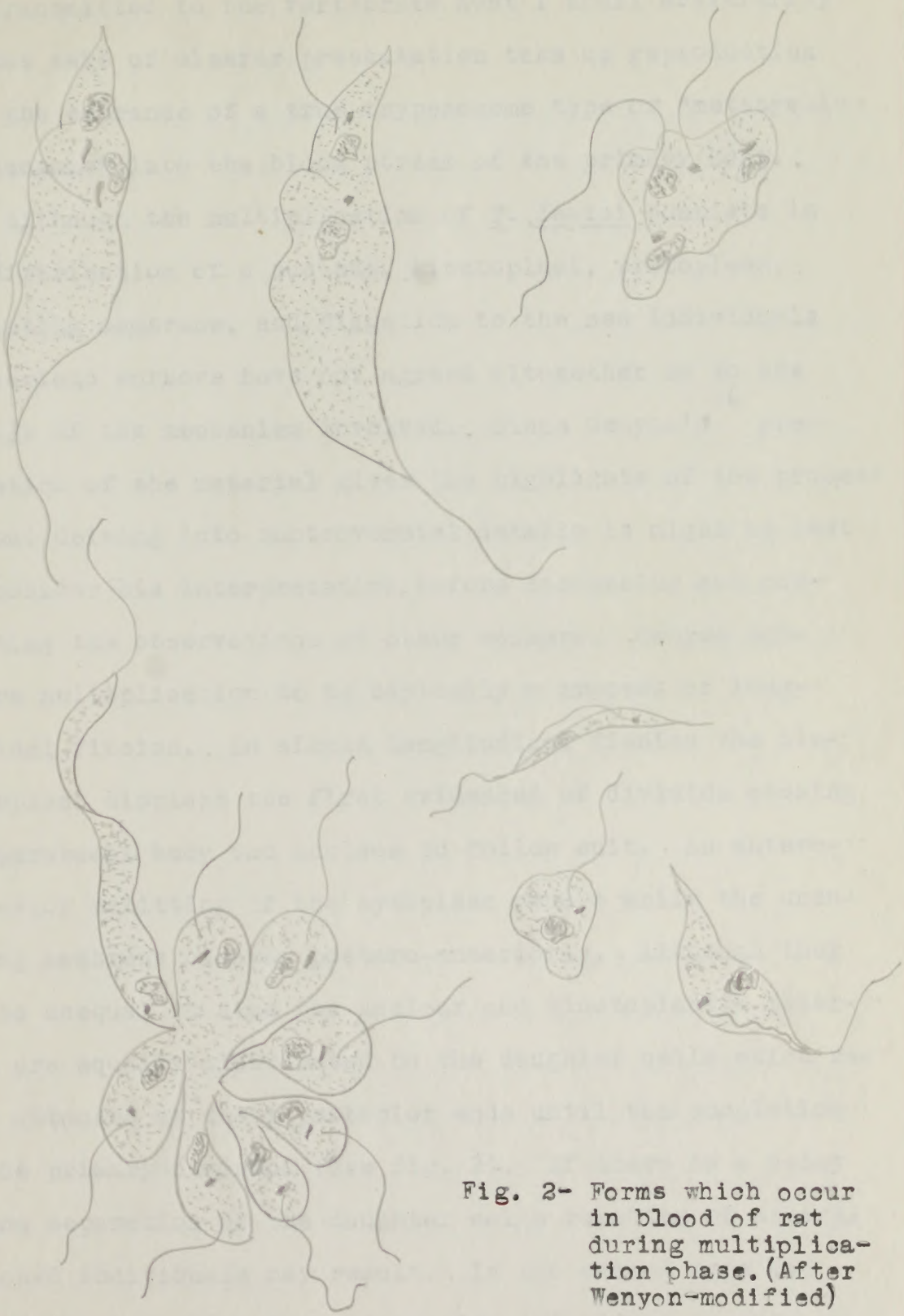

Fig. 2- Forms which occur in blood of rat during multiplication phase. (After Wenyon-modified)

are transmitted to the vertebrate host I shall arbitrarily for the sake of clearer presentation take up reproduction with the entrance of a true trypanosome type or "metacyclic trypanosome" into the blood stream of the primary host.

Although the multiplication of T. lewisi consists in the distribution of a nucleus, kinetoplast, protoplasm, undulating membrane, and flagellum to the new individuals the various workers have not agreed altogether as to the details of the mechanism involved. Since Wenyon's[54] presentation of the material gives the highlights of the process without delving into controversial details it might be best to consider his interpretation before discussing and correlating the observations of other workers. Wenyon considers multiplication to be basically a process of longitudinal fission. In simple longitudinal fission the blepharoplast displays the first evidences of division causing the parabasal body and nucleus to follow suit. An anteroposterior splitting of the cytoplasm occurs while the undulating membrane cleaves postero-anteriorly. Although they may be unequal in size the nuclear and kinetoplastic materials are equally distributed to the daughter cells which remain attached at their posterior ends until the completion of the primary division (See fig. 2). If there is a delay in the separation of the daughter cells rosettes of several attached individuals may result. In the second type of longitudinal fission the multiplying individual assumes an

intracellular, multinucleate condition, with the fromation of a corresponding number of kinetoplasts with attached flagella, before the onset of cytoplasmic fission (See fig.3).

Essentially, Laveran and Mesnil[28] explain multiplication as does Wenyon. They divide multiplication forms into two groups, the first being unequal longitudinal division and the second corresponding to the intracellular type described by Wenyon. Multiplation by unequal longitudinal fission occurs after: the enlargement and division of the nucleus and kinetoplast, the latter two structures becoming closely apposed; the thickening and splitting of the flagellar root; and cytoplasmic fission. Sometimes both the small and large daughter cells subdivide giving rise to rosettes containing unequal individuals. The second type is the formation of several nuclei, kinetoplasts and flagella before cytoplasmic division. The fact that a larger parent trypanosome can not be distinguished is the characteristic feature of the latter method. MacNeal,[31] however, contends that the second method of Laveran and Mesnil does not occur, the larger, mother cell persisting as a visible structure. But, in summing up their second method of reproduction Laveran and Mesnil grant the possibility that it is derived from the first, the smaller of the two individuals resulting from unequal division giving rise to a number of nuclei and kinetoplasts with a delay in divisions of the whole body. Actually MacNeal and the two French workers differ

only in interpretation since the latter admit the great possibility of an intracellular multinucleated type stemming from the smaller daughter cell resulting from unequal longitudinal division. If, in the hypothesis advanced by Laveran and Mesnil the stem cell of the multinucleated type were to remain attached to its larger, sister cell while generating a number of small trypanosomes one would then have the situation insisted upon by MacNeal since the larger mother cell would then be visible. Since the terms "mother" and "sister" cell may be confusing -- the difference between the two is merely a matter of time. Before division a trypanosome ready to multiply would be known as a mother cell. When the first division has taken place the mother cell has given rise to a smaller trypanosome known as a daughter cell, while the larger cell may be called a mother or daughter cell depending upon the phase of multiplication being considered. Additional cells resulting from further divisions of the two would also be called daughter cells.

In his classification of multiplication Francis[18] offers three methods: transverse fission, longitudinal fission, and segmentation. After the formation of several nuclei, kinetoplasts and flagella the body divides at right angles to the longitudinal axis in the case of transverse division, while in longitudinal fission the plane of division is within the longitudinal axis of the organism.

Longitudinal and transverse fission are most likely the same mechanism, the difference lying simply in axial relationship. The third method described by Francis introduces a new element characterized by fusion of the ends of an individual, the assumption of a globular from and the disintegration of flagellum and undulating membrane. Nuclear and kinetoplastic multiplication ensue with cleavage occurring from the periphery inwards. Except for the fusion of the two ends the latter method of Francis follows the pattern of intracellular multinucleation previously discussed.

Longitudinal fission, then, may be said to be the general procedure in multiplication within the vertebrate host. The observations and hypotheses of the leading investigators although set forth as distinct entities can be integrated and are, in my opinion, different aspects of a fundamental principle.

Because of their close association the kinetoplast, flagellum, undulating membrane and nucleus during the process of multiplication will now be discussed. After enlargement of the body in all dimensions the kinetoplast in the event of unequal longitudinal fission is the first structure to divide.[54] MacNeal,[31] however, says that nuclear division is occasionally the first symptom of fission. Both MacNeal and Francis agree that a flagellum arises anew from the daughter kinetoplasts, but Laveran and Mesnil[28] state that a thickening of the flagellar base occurs with

its consequent splitting into two. Taking a position posterior to the kinetoplast the nucleus then divides. According to MacNeal[31] the daughter organism possesses an undulating membrane in the form of a "rudimentary fold of outer protoplasm". In the intracellular, multinucleate condition small flagella are generated from the kinetoplast and their lashing aids in cytoplasmic division. The resulting organisms resemble the adult trypanosome but are oval and in comparison are greatly reduced in size. During the period following cytoplasmic cleavage they appear in various stages but are typically crithidial in morphology, the kinetoplast being anterior to the nucleus. A gradual transformation to the adult form takes place.[28] MacNeal[31] states that nine to ten days are required for completion of the multiplication stage since at the end of that time adult forms (See fig. 3) only can be found in the blood of a white rat previously injected with blood containing multiplication forms. It will be later pointed out that a specific antibody is produced by the host which is responsible for the cessation of multiplication at the end of nine or ten days.

For a number of years after Lewis' work on the rat trypanosome investigators were ignorant as to its mode of transmission. The multiplication phase in the rat had been studied but how the parasite was transferred from rat to rat remained a mystery. Numerous speculations and

Fig. 3- Adult forms in the blood of the rat.
(After Wenyon-modified)

hypotheses were advanced while the term, "spontaneous infection", is typical of the expressions used to represent the missing link in the life-history of T. lewisi. Ceratophyllus fasciatus, the rat flea, was proven by Rabinowitsch and Kempner[41] to be the true vector of rat trypanosomiasis, in an admirable paper published in 1899. Several lines of investigation were followed until they decided to confine their research to a possible insect vector.

Although Francis[18] had produced trypanosomiasis by the feeding and intrastomacheal injection of infected blood to previously uninfected white rats, Rabinowitsch and Kempner eliminated the alimentary tract as a portal of entry for infected blood on the evidence of several experiments. Intrastomacheal injection of infected blood and the feeding of an infected rat to an uninfected rat were unsuccessful. It is interesting to note that a later investigator eliminated the possibility of trypanosome transmission by a lactating rat to the sucking young.[16] In another experiment an infected tame rat was placed in the same cage with an uninfected wild rat. After a heated battle the tame rat was killed and eaten by the wild rat which in ten days showed trypanosomes in blood smears. Rabinowitsch and Kempner maintain that the bite-wounds inflicted upon each other by the rats provided a direct portal of entry for the infected blood shed by the tame rat

which came into contact with the fresh wounds of the wild rat. The digestive tract, then, was still eliminated. Non-infected and infected white rats were placed together in a cage and after eleven days during which the rats got along with each other, no bite-wounds being apparent, the previously uninfected rats contained T. lewisi in its blood. At this stage of the work it was decided to seek a possible insect vector on the instigation of Dr. Robert Koch since the relation of insects to the transmission of certain pathogenic microorganisms had been demonstrated in research on such diseases as Texas Fever, malaria, and nagana. The co-workers found both wild and tame infected rats harboring fleas and lice containing miniature trypanosomes; a preparation of flea visceral material in physiological saline injected intraperitoneally into a white rat produced an infection within eight days. A similar experiment with louse material was unsuccessful so that attention was devoted to the flea which at that time was considered to suck up and harbor parasites present in infected blood. Although the true mechanism of transmission was later discovered by other workers, Rabinowitsch and Kempner believed that it was accomplished by the means of piercing mouth-parts of the flea. In an experiment considered positive proof that the flea transmits T. lewisi about twenty fleas taken from an infected rat produced an infection when transferred to a previously uninfected rat.

There now remained two gaps in the knowledge of the life-history of T. lewisi- - the actual method in which the parasite was transferred from flea to rat and its maturation within the flea. Wenyon in 1913[53] and Noller[37] in 1912 showed that transmission is accomplished by the rat licking off moist faeces of infective fleas containing the final form of the developmental cycle. In his research Wenyon collected infected faeces in a fine pipette and by transferring the diluted faeces to the rat's mouth was able to cause an infection. Mere feeding of the flea upon a rat without the ingestion of faeces was unsuccessful. Minchin and Thompson[33] confirmed the latter's results by experiments in which flea-infested rats were muzzled to prevent licking of flea excreta.

Immature forms of T. lewisi had been observed in visceral mounts of the rat flea, but a detailed, accurate description of the developmental cycle within the flea was not available until the publication of Minchin and Thompson's paper[33] in 1915. The result of several years' research the purpose of the undertaking was "to furnish a standard with which life-histories of other species of trypanosomes might be compared..."

After the first blood-meal was ingested by the rat flea, five or six days were required for it to become infective; therefore, it was deduced that the developmental cycle occupied the same period of time. Essentially, the

metamorphosis of the trypanosome in the flea consists in the development of a crithidiomorphic individual in the stomach and the transformation to a crithidial and subsequently trypaniform organism in the rectum. Blood containing adult trypanosomes passes to the stomach where the posterior third of the cell body becomes rigid. This is believed due to absorption of blood altered by gastric digestive processes. There is a tendency to attach to the gastric epithelium by means of the flagellum which is inserted between two epithelial cells, the body lying lengthwise along the stomach lining. Following the penetration of an epithelial cell by the trypanosome a sphere or vacuole is formed in which the parasite may be very motile and experience various morphological changes. Within the vacuole multiple nuclear and kinetoplastic fission occurs and stiff crithidiomorphic individuals characterized by the anterior position of the nucleus in relation to the kinetoplast are liberated into the stomach by the rupture of the epithelial cell and migrate to the rectum. A transformation to a true crithidial type (kinetoplast anterior to nucleus) which either multiplies by binary fission or develops into a small trypaniform individual occurs during the rectal phase of the cycle in which many variations from the true crithidia to the small trypanosome type may be found. It is to be stressed that the trypaniform individual exhibits the adult arrangement of kinetoplast and

nucleus and constitutes the end-point of the cycle in the intermediate flea host. Only faeces containing this final developmental stage will be infective to the rat (See fig. 4).

The exact significance of passage through an active insect vector besides that of reproduction has not been ascertained. It may be a rejuvenatory function with a resulting increase in virulence or on the other hand an attenuation may be effected. That a change of properties occurs is certain. Gonder[22] working with arsenic-fast strains of T. lewisi found that this property is maintained by artificial subinoculation. However, passage through the rat-louse, Haematopinus spinulosus, brings this arsenic-fastness to an end. Investigation of the physiological chemistry concerned in the relationship of the trypanosome and its insect host may, when properly correlated, lead to the discovery of a valuable specific against human trypanosomiasis. As was stated earlier the complexity of the insect phase points to the probable ancestry of mammalian trypanosomes, since the cycle in the latter is comparatively simple.

An interesting sidelight on the biology of T. lewisi in the rat louse has been thrown by Prowazek[40] who claims therein the existence of sexual phenomena. Development in the louse is considered by him to begin with a process of fertilization. After the differentiation of slender male and stout female forms a nuclear chromosome reduction

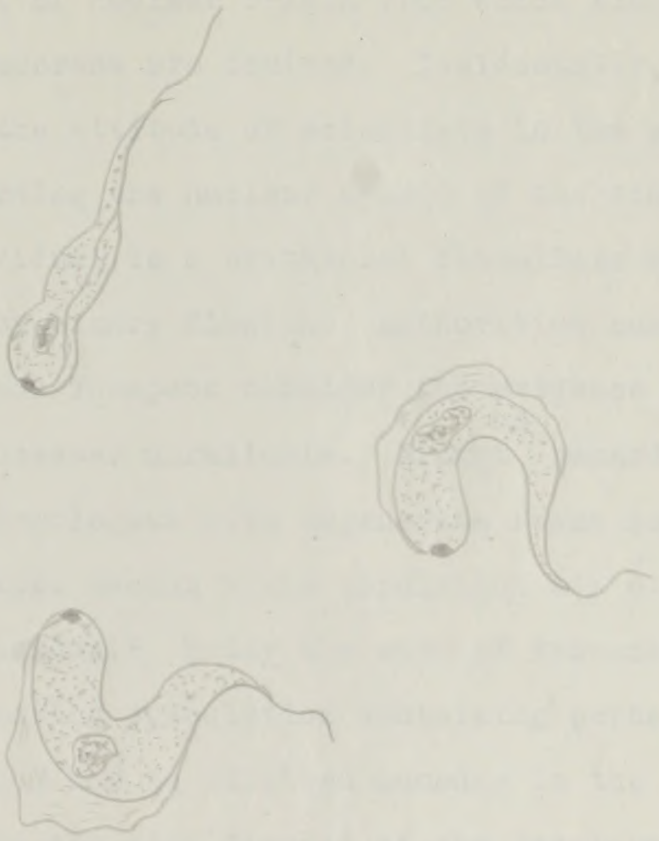

Fig. 4- Metacyclic forms found in the faeces of infective fleas. (After Wenyon-modified, enlarged)

occurs followed by nuclear fusion or fertilization. A "zygote" without a flagellum results and proceeds to produce a kinetoplast of nuclear origin from which flagellum and undulating membrane are derived. Incidentally, this illustrates the attitude of scientists in the early twentieth century regarding the nuclear origin of the kinetoplast. The new individual is a crithidial flagellate which proceeds to multiply by binary fission. Authorities such as Wenyon and Minchin and Thompson consider the evidence for existence of sexual processes unreliable. Brumpt[8] regards the male forms to be homologous with degenerate forms in the infusoria: " l'heure actuel aucune vraie copulation n'a été vue chez les trypanosomides." Today the work of Prowazed remains a highly interesting speculation containing perhaps a germ of actuality which may be utilized someday in the further elucidation of the significance of the developmental cycle of trypanosomes in the insect vector.

In summary, it might be well to state briefly the basic facts described by Minchin and Thompson and other workers regarding the transmission of rat trypanosomiasis. The chief transmitting agent, the rat flea, is an active, biological vector; although the rat louse has been mentioned as a possibility it is believed to be a passive, mechanical vector, infection being produced by ingestion of a louse containing infected blood in the gut. The salivary glands of the flea are not penetrated by trypanosomes, the only organ system to harbor them being the alimentary tract.

Infection is brought about by the small trypanosome or final metacyclic type in the flea cycle. Therefore, infection can be achieved only upon completion of the developmental cycle when the rat licks moist flea faeces containing the diminutive trypanosomes. Ingestion of flea faeces by the rat before the completion of the cycle cannot produce infection.

IV. VIRULENCE AND IMMUNOLOGY

Agglutination

Once the trypanosome has invaded the body of the rat via an acceptable portal of entry it multiplies, liberates toxic materials, and since it represents a foreign protein substance is combatted by the defensive mechanisms of the rat. When immunological investigations of T. lewisi were undertaken several phenomena were observed which pointed to the existence of antigen-antibody reactions. Since rapid advances were being made in bacteriology at the same time, men working with trypanosome immunology had the benefit of the discoveries being made in the former science. Agglutination, especially, was the phenomenon first studied by the early investigators of the rat trypanosome.

The severity of an infection and heaviness of infestation are not necessarily directly proportional. A relatively greater number of trypanosomes has not been observed by Laveran and Mesnil[28] in blood smears made from white rats dying of a virulent strain. Francis,[18] however, reporting that many of his white rats died of trypanosomiasis states that a heavy infection is sure to cause death. According to him an infection in white rats lasts from seven to fourteen days and in wild rats is indefinite. Naiman[35] discovered (1944) that X-irradiation of rats increased the

severity of infection from two to eight times. Pathogenicity among rat trypanosomiasis is not usually encountered, but Roudsky[44] working with virulent strains developed in the laboratory observed degenerative changes in white mice with organ enlargement, lymphoid infiltration in liver and spleen, and lung congestion.

When mixed with trypanosome blood the serum of rats recovered from an infection has the ability to agglutinate the organisms, but this particular type of agglutination is unlike the bacterial variety in that motility is retained by the individuals forming the resulting configuration known as a "rosette". Flagella and undulating membranes of agglutinated individuals actively lash and move about. Agglutinated organisms have been seen to separate, but if agglutination persists the trypanosomes may degenerate. It seems, therefore, that two immune factors are involved, an agglutinin and a trypanolysin to state the opinion of Laveran and Mesnil.[27] In rare cases, MacNeal[31] has observed the complete disappearance of parasites following agglutination and believes it an immune reaction of the host. Francis[18] has seen autoagglutination in the blood of infected rats. According to him it occurs after the height of infection has been reached and is evidence for the presence of active immunity, the insufficient production of agglutinins being held responsible for instances in which autoagglutination does not occur. It would be logical,

therefore, to assume that variations in the virulence of the organism and the defensive ability of the host cause this inconsistency in the frequency of autoagglutination, since a comparatively virulent organism would be required to stimulate the formation of sufficient agglutinins to bring about rosette formation.

Ignoring the possibility of autoagglutination Laveran and Mesnil[27] consider that two distinct conditions will cause agglutination: the maintenance of defibrinated blood in cold temperatures and the contact of trypanosome material with the sera of certain animals or infected rats. The latter reaction is much more rapid and because of the thermolability of the agglutinating substance is believed due to presence of agglutinins. The fact that both cryogenic methods and antibody formation initiate an agglutinating process thought to be an indication of immunity points to metabolic inhibition or degeneration, cold temperatures being a standard means for depressing the metabolism of microorganisms. Therefore, agglutination must be a mechanism of the host body used to lower the vitality of the invading organism prior to the establishment of immunity. It will be shown later that agglutination is but the first of a series of reactions which result in the destruction of the parasites and which are now believed to be caused by a single antibody. Disagglutination or "disagglomération", to use the term employed in French

papers, occurs when the trypanosomes are motile enough to escape from the rosette - - in other words, they are of a vitality strong enough to resist and overcome the action of the antibody or rather, possess a virulence powerful enough to repel the host defensive reactions.

In his account of agglutination MacNeal states that after the ninth or tenth day of infection when multiplication has ceased the posterior tips of the adult trypanosomes get sticky so that they easily adhere to blood corpuscles or to one another. There is no loss of motility, the organisms joining at their posterior tips to form rosettes composed of a varying number of individuals. Laveran and Mesnil[27] have seen platelets or leucocytes forming the focus of a rosette. Sometimes several rosettes are associated in a system composed of a rosette forming a primary focus with several satellite rosettes constituting secondary foci of agglutination. Crowded masses of the agglutinated parasites overlapping each other, may, according to MacNeal give the impression that conjugation occurs; he probably mentioned this fact with the sexual phenomena claimed by Prowazed in mind.

Immunity - Active and Passive

The agglutinating property of rat serum is produced by successive inoculations of infected blood into a normal uninfected rat.[27] An active immunity, therefore, is brought

about. Laveran and Mesnil who accomplished much valuable work on this aspect of the subject found that five to ten intraperitoneal inoculations of trypanosome blood will produce a serum with an agglutinating value ranging from five to fifty. A value higher than ten will cause persistence of agglutination. It was also discovered that a paralysin is formed as a host immune reaction, but is found only in sera of rats injected many times with trypanosome blood. As has been stated before the paralyzing reaction is a more advanced phase of the immune reaction which begins with agglutination and is due to a single antibody. However, the early investigators believed that "agglutinins" and "paralysins" were a distinct entity. Sera visibly containing "paralysins" or to say it more correctly, sera of a sufficient strength to bring about paralysis were sometimes so paralyzing that a serum dilution of one in ten prevented rosette formation. That is, the paralytic power of the serum was so rapid and overwhelming that the trypanosomes were immobilized before rosette fromation or the normal sequence of the immune reaction could be accomplished. Thermolability of the agglutinins was demonstrated by both Laveran and Mesnil[27] and Francis[18] who either attenuated or destroyed the agglutinating property of a serum by subjecting it to a temperature of 65°C for a half hour. Although the agglutinating property was destroyed no mention has been

made in the literature of the thermolability of the other antibody which has not yet been discussed.

It was discovered that the sera of other animals unexposed to T. lewisi antigens have been found to harbor substances capable of agglutinating T. lewisi. Francis[18] using a 1:1 dilution found that goat and rabbit sera are weakly agglutinating; Laveran and Mesnil[27] using sheep, goat, and rat sera found that they also possess a relatively weak aggluting power which varies in strength. Fowl, horse and cat sera are strongly agglutinating, while Francis states that the sera of white rats, mice and guinea pigs are negative when unexposed to T. lewisi antigens. The sera capable of agglutinating trypanosomes had the same action on erythrocytes in the infected material used for agglutination tests. Incidentally, the agglutinating property is not affected by low temperatures, Francis having found rosettes after 83 days in refrigerated blood drawn aseptically from an infected rat and sealed in pipettes. Rosette fromation being the first visible evidence that immune reactions are taking place and that the vitality of the microorganisms is being impaired one would naturally wonder if agglutinated trypanosomes have the virulence to reestablish an infectious process when transferred to a normal rat; they do. Agglutinated preparations have been found to be as infective as unagglutinated material injected as control. Since a previously uninfected host would not yet have adjust-

ed itself to trypanosome antigens the organism would have little difficulty in regaining the virulence needed to incubate and multiply in the fresh blood medium of the new host. Laveran and Mesnil[28] report that rosettes belonging to secondary foci of agglutination are active while the central rosettes die quickly in the event that masses of primary and secondary rosette foci are formed. It seems likely that an accumulation of toxic waste products occurring in the inner region might cause degeneration of central rosettes while those individuals in the outer ring of secondary agglutination masses survive because of unhindered exchange of waste products with the medium.

Rabinowitsch and Kempner[41] found that a rat recovered from a first infection could never be reinfected and assume therefore, that an active immunity had resulted. The existence of active immunity was also suggested by Francis[18] whose white rats tolerated a second infection for only three days. The young born of immunized females are susceptible to infection maintain Laveran and Mesnil, since the placental barrier prevents both antigen and antibody from passing into the foetal circulation. However, recent work on transmission of immunity to the young has altered the older concepts. Culbertson (1941)[12] actively immunized pregnant female rats by the administration of a formalized suspension of T. lewisi. It was found that antibodies are transferred to the sucklings in the process

of lactation. However, the immunity conferred upon the young is of short duration and is lost within a few weeks after weaning. The young born of immunized females were found to be susceptible to infection in the short period following birth when not allowed to suckle on their mothers. Although the placental barrier is commonly considered a definite barrier to antibodies, Culbertson states that any immunity which may be acquired through the placental circulation is lost within a few days after birth. The reproduction-inhibiting factor (discussed below) is believed responsible for the temporary immunity of the young which are allowed to suckle.

In cases of active immunity the two French workers consider leucocyte phagocytosis the only method of trypanosome destruction for when trypanosomes are injected intraperitoneally into an actively immunized rat they rarely appear in the blood and are considered to be engulfed by leucocytes present in peritoneal fluid. Examination of peritoneal fluid revealed the presence of many leucocytes, 2/3 granulocytes and 1/3 agranulocytes. The parasites were actually seen in the process of being swallowed by the white blood cells. Control experiments were made with normal rats and phagocytosis does not occur. No mention has been made of phagocytosis of trypanosomes occurring in the blood stream. It doubtlessly does occur. Duca[15] records the presence of leucocytosis and "marked

absolute monocytosis" as part of the blood reaction. The ultimate fate of trypanosomes in the rat will be further discussed below.

Since existence of active immune phenomena has been demonstrated it is not surprising that homologous immune serum has a protective value in T. lewisi infections. Experiments utilizing techniques to bring about passive immunity to T. lewisi infections in the rat have met with success. Francis[18] states that immune serum injected into a rat enhances the refractory properties of the organism so that the severity of an infection is reduced or the parasites disappear completely. Combining one cubic centimenter of homologous immune serum with an equal amount of trypanosome blood, Francis injected the mixture into a fresh rat; no infection resulted. He also discovered that the same amount of immune serum injected twenty-four hours before or after injection of trypanosome blood prevents infection. However, this technique of passive immunization is confined to time limits for once the parasite entrenches itself in the host injection of immune serum is useless since the only resistance to the organism will be provided by the production of antibodies, a gradual process. However, Laveran and Mesnil[28] have inoculated rats with immune serum during the second week following injection of trypanosome blood. About half were refractory; the others contracted light infections of short duration.

Immune serum for use in passive immunization is produced by repeated injection of infective blood until the desirable level of antibody production is reached. Laveran and Mesnil found that the most potent sera were obtained from rats which had received thirteen and ten injections of infected blood respectively. A figure lying between ten and thirteen probably would indicate the stage at which the most desirable serum is manufactured. Although X-irradiation decreases resistance to T. lewisi previous exposure to X-rays does not diminish the effect of subsequent treatment with immune serum. From a general acquaintance with the immune reactions concerned in the rat trypanosomiasis one would be prone to believe that paralysins or more probably trypanolysins are responsible for the final destruction of the parasite when active or passive immunity is achieved. Laveran and Mesnil noted that agglutination and paralysis can dispose of the micro-organisms in vitro when immune serum and trypanosome blood are used, but these properties were not considered by them to play the leading part in immunity because of their thermolability. They believed that a leucocyte stimulation results in leucocyte phagocytosis of the trypanosomes. Their view is based on periodic examinations of peritoneal fluid after intraperitoneal injections of trypanosome blood. Although a slight agglutination was observed, final destruction is, according to them, accomplished by phagocytosis in the

peritoneum - - when the portal of entry is intraperitoneal.

MacNeal[18] disagrees with the two French workers in that he assigns the chief role to cytolytic agents rather than phagocytosis. The interpretation given to phagocytosis by Laveran and Mesnil is interesting. They believe passive immunity to be of a "cellular" rather than a "humoral" nature, to use terms which are now obsolete. When one considers that one of the effects of the single antibody concerned in the destruction of the rat trypanosome is most likely opsonic one must concede that in this case immunity is "humeral". It is very likely that Laveran and Mesnil were unfamiliar with the work on opsonins since they would then be aware of the reason for leucocyte stimulation, an antibody, which would in their terminology be of a "humoral" nature.

In attempts to treat rats with well established infections Laveran and Mesnil used various substances. Four cubic centimeters of serum from immunized rats were injected with results ranging from complete disappearance of the parasite to no diminution at all. Treatment with sodium arsenite, trypan-red and human serum were unsuccessful. It was found that the protective properites of immune serum could be attenuated by heating to 64°C for one-half or three-quarters of an hour. If we accept the hypothesis that the trypanocidal antibody is extremely complex in view of the multiple effects it has on both parasite and

and host (i.e. increased leucocytosis) it can be assumed that one of the elements in the structure of the antibody is an opsonin and would therefore be sensitive to the heat; this can be explained in the light of present-day knowledge regarding opsonins. There are, apparently, two distinct substances associated in opsonin structure - - a heat resistant factor which largely performs the immune function and seems to be activated by the presence of the other, thermolabile factor.[25] Destruction of the latter by heating inactivates the more important thermo-resistant element and, therefore, destroys or reduces the protective attributes of an immune serum.

As has been stated previously, the early workers considered separate antibodies to be responsible for agglutination, paralysis and lysis, but recent work has led to the theory that a single trypanocidal antibody produces the gamut of reactions, including opsonin formation, formerly held to be caused by distinct immune substances. I have been unable to find definite proof in the literature in favor of the modern view which may be closer to the truth since leading parasitologists support it. Investigations of the host reactions elicited by T. lewisi have been carried on during the last twenty-five years and a more complete picture of the antibodies involved and the final destruction of trypanosomes is now available.

Taliaferro[47] is 1923 showed that rat serum when the

reproductive stage has ceased contains a reproduction-inhibiting substance which does not destroy the parasite. This was demonstrated by the intravenous injection of washed adult trypanosomes with serum containing the inhibitory substance. There were no signs of reproduction in subsequent blood smears. Delving further into the nature of the reproductive-inhibitory antibody Coventry[9] in 1925 could not detect the substance before the fifth day following injection of trypanosome blood. There is an increase in the reaction product titre between the fifth and sixth days, the peak in titre being attained on the 35th day, whereupon it decreases as the infection peters out. It has since been named, "ablastin".

As to the final disposal of the parasites present-day workers do not quite agree with the older concept of Laveran and Mesnil regarding "Leucocyte phagocytosis", although phagocytosis is assigned the function of ridding the rat of the parasite. D. L. Augustine,[3] a leading authority, states that the microorganisms are destroyed in the liver and probably in the spleen. There is definite proof that malarial parasites are destroyed in the spleen.[32] Therefore, the macrophages of the liver and spleen may be said to phagocytize the trypanosomes. However, there is conflicting evidence on the subject of spleen macrophages in T. lewisi infections. For example, Schwartz[46] in 1931 stated that splenectomy has no appriciable effect on the progress of a

trypanosome infection in the rat, although one would logically expect decreased resistance to infection to result from removal of the spleen. In the same year, Taliaferro, Cannon, and Goodloe[48] presented seemingly overwhelming evidence in favor of the spleen theory. They also demonstrated the importance of the spleen in the manufacture of ablastin. When splenectomy was performed the same day as the injection of a rat with T. lewisi, production of ablastin was so impaired that multiplying forms could be seen in the blood for 13 to 30 days whereas ten days is the maxium time in which division forms may be seen in normal rats. Since T. lewisi infections last longer in splenectomized rats the spleen is regarded by the three workers as an important source of the trypanocidal antibodies. Since the spleen contains a goodly number of the body macrophages its removal would seriously diminish the phagocytic potential of the rat and it is probable that the latter condition rather than decreased trypanocide production is responsible for longer infections resulting from splenectomy.

The evidence for and against the role of the spleen having been presented a correlation of the two opposing views is offered by Maximow and Bloom.[32] They believe conflicting evidence regarding the question of splenectomy to be due to several factors: "variations in different species of the spleen-weight to body-weight ratio...compensatory action of other organs after one or two weeks (eg bone

marrow and liver).

In summary, it is now believed that two antibodies are involved in T. lewisi infections: ablastin, a reproductory-inhibiting antibody, and a trypanocidal antibody. The latter exerts a progressive series of reactions upon the rat trypanosome which ultimately results in the parasite's destruction. This same antibody is interesting because it performs immunological functions usually associated with separate antibodies rather than just one; it is responsible for agglutination, paralysis, lysis, and opsonic phenomena.

V. RODENT TRYPANOSOMES

In a discussion of trypanosomiasis the rat is usually presented as a representative example of an infected member of the rodent family. Constituting a world-wide source of material comparatively readily obtainable for study the rat has attracted the bulk of scientific attention. For that reason parasitologists are prone to disregard other rodent hosts of trypanosomes which, although harder to obtain than the rat, do, nevertheless, exist and should, therefore, be considered in this paper.

In a trypanosomiasis expedition to Senegambia early in the twentieth century Dutton and Todd[28] discovered trypanosomes in the blood of mice (*Mus muris, M. musculus*) on MacCarthy Island in the Gambia River. Since named *Trypanosoma duttoni* the organism according to the two investigators did not possess an undulating membrane nor was any pathogenicity apparent. Three out of fourteen examined were positive, an incidence of 21%. Rats inoculated with infected blood were refractory. Resembling *T. lewisi*, the organism can be subinoculated from mouse to mouse while Brumpt[8] in 1913 stated that *T. duttoni* undergoes a developmental cycle in the swallow-flea, *Ceratophyllus hirudinis*, similar to that experienced by *T. lewisi* in the rat flea and that infection is brought about only by ingestion of faeces containing the small or metacyclic

trypanosomes. He also found developmental forms in the bedbug (Cimex lectularius), but it is doubtful if the latter is the chief vector if it is a vector at all of the mouse trypanosome; mere content of trypanosomes does not necessarily mean that they are transmitted to a vertebrate. In experiments similar to those carried out by Wenyon[53] with rats and the rat flea, Brumpt, working with mice several days old (since they are more susceptible), produced infections by oral administration of faecal, swallow-flea material as well as by the intraperitoneal route. Injected material was first examined for the presence of the trypaniform individuals. Subcutaneous injection and rectal administration of infective faeces were unsuccessful.

Discovered by de Nabias and Jolyet[24] at Bordeaux in a rabbit suffering with a "suppurative otitis" the rabbit trypanosome has been found in Lepus domesticus and L. cuniculus. It was encountered again during the preparation of a white blood count and was at first believed to be a microfilaria, but further study uncovered its true identity. According to them the rabbit trypanosome, T. nabiasi, measures 30 to 36 micra in length and 2 to 3 micra in width. It seems that infected rabbits examined by Jolyet and de Nabias exhibited diminished vitality, a symptom which may be indicative of pathology or may be entirely coincidental. In his investigation of T. nabiasi Brumpt[8] proved that a developmental cycle occurs in the rabbit flea, Spilopsyllus

cuniculi. Both development and transmission are identical to that of T. lewisi in Ceratophyllus fasciatus. No cycle was found by him in Cimex lectularius. Four rabbits of 29 examined were positive while uninfected animals were immune according to the results of eight experiments. Immunity in this instance may be active due to previous infection and recovery, however, it does seem strange that subinoculation was unsuccessful.

Kunstler[26] claims the existence of a guinea-pig trypanosome, only a few out of several hundred animals examined having been positive. From the description and illustrations of the microorganism Laveran and Mesnil, and Wenyon do not consider it to be a trypanosome at all; the French workers believe it to be a trypanoplasm.

T. rabinowitschi, the hamster trypanosome, was first seen by Koch and Von Wittich[28] in 1881 in the blood of Cricetus frumentarius. In their studies on this parasite Rabinowitsch and Kempner[41] could hardly distinguish it from T. lewisi and concluded that the hamster and rat trypanosomes are two varieties of the same species; however, the former could not be transferred to the rat by intraperitoneal injection. Investigating the immunological implications of these so-called "varieties" the hamster parasite was unsuccessfully injected into tame rats; consequent injections of T. lewisi showed some of them to be refractory to it. If other rats had not developed T. lewisi

infections one might have concluded that immune reactions were set up against the hamster trypanosome which were equally efficacious against the rat parasite, both, supposedly being varieties of the same species and having similar antigens. However, it will be pointed out below that such a condition is a great possibility. As was the case with previously discussed trypanosomes Brumpt[8] examined the hamster flea, Typhlopsylla canis and found trypaniform individuals in the rectal contents. From the evidence of unsuccessful subinoculation into the rat I believe it safe to say that the rat and hamster trypanosomes are distinctly separate species rather than varieties, despite the morphological resemblances. However, a common ancestry of both trypanosomes is not out of the question as will be shown later.

The first research on the trypanosome of the gopher or spermophile was done by Chalachnikov[28] in Russia. Working with Spermophilus musicus and S. guttatus he found a 12% parasitization and successfully cultured the gopher trypanosome which has since been named T. spermophili. Measurements of length and width taken by Chalachnikov are 30 to 40 micra and one micron respectively.

Sciurus palmarum, the Indian squirrel was found in 1903 to harbor a trypanosome.[28] Dr. C. Donovan obtained an incidence of about 22 percent in Madras. According to Laveran and Mesnil slides sent them by Donovan contained only thin,

adult forms, no multiplication forms being visible. Rather than the anterior location of the T. lewisi nucleus, that of T. indicum, as this trypanosome has been named, is centrally situated, while the kinetoplast is especially large and striking. Laveran and Mesnil regard it as a distinctly new species of trypanosome.

The trypanosome of the dormouse, Myoxus avellanarius, T. myoxi, has been seen only in hanging drop. Galli-Valerio noted[21] the presence of flagellum and undulating membrane, the length being about 22 micra. His preparations were made from the heart's blood and the morphological characteristics led him to believe the parasite to be a trypanosome. T. blanchardi was discovered by Brumpt[8] in the garden dormouse, Myoxus nitela. A developmental cycle identical with that of T. lewisi in the rat flea has been demonstrated in the dormouse flea, Ceratophyllus laverani; Brumpt transmitted the infection using this flea. Wenyon believes T. blanchardi to be the same as that seen by Galli-Valerio.

Blood smears made from a number of other rodents has revealed the presence of trypanosomes. There is but a cursory mention of the following hosts and parasites by Wenyon,[54] no further references being available. T. avicularis, a T. lewisi type was discovered in the Sudan by Wenyon in the zebra-mouse, Arvicanthus zebrae. T. acomys discovered by him in the Sudan is a parasite of the spiny-mouse(Acomys-sp.). Laveran and Pettit found T. grosi in the field-mouse,

Mus sylvaticus, and were unsuccessful in attempts to sub-inoculate it to other animals; they also found T. microti in the field vole, Microtus arvalis.

In Canada Hadwen[51] discovered a trypanosome, T. evotomys in the field vole, Evotomys saturatus; compared to T. lewisi it has a more centrally located nucleus and is non-pathogenic for rabbits and guinea-pigs. In Canada also, Peromyscus maniculatus and P. nebrascencis have been found to contain T. peromysci. Watson,[51] the discoverer, found a 20% infection and noted that the parasites disappear from the blood of mice held in captivity on the second or third day; the organisms are non-pathogenic.

Brimont[5] in French Guiana discovered a trypanosome in the agouti (Myoprocta acouchy)- T. accouchii, a T. lewisi type. In the Californian ground squirrel, Otospermophilus beecheyi, Wellman and Wherry[52] found T. otospermophili, which they say has a superficial resemblance to the rat trypanosome since intra-peritoneal injection of physiological salt solution containing lung emulsion to two white rats and a guinea pig were unsuccessful. They decided a new species had been discovered since T. lewisi is so readily inoculable to rats.

Lingard[30] in India discovered T. bandicotti in the blood of the bandicoot, Nesokia gigantea; it resembles T. lewisi and is inoculable to guinea-pigs in which it is fatal. The parasite is usually absent in full-grown animals

but when found was present in young, half-grown animals.

The occurrence of trypanosomes in a wide range of rodents and the usually low incidence of infection serves to support the premise previously mentioned that they were originally insect parasites since insects, especially fleas, are always found in relation with trypanosome-infected rodents. Great significance may be seen in the variety and distribution of rodent trypanosomes, many of which resemble T. lewisi closely. The possibility that they are all derived from a common, ancestral trypanosome will be considered below.

VI. EXPERIMENTATION AND CULTURE

Maintenance in Laboratory Animals

Since wild rats do not thrive well and usually succumb to captivity it is convenient to subinoculate the rat trypanosome into a susceptible laboratory rodent or maintain it *in vitro* so that prolonged observation and study may be carried on. Almost always *T. lewisi* is non-pathogenic to rats since it is a natural host. On the subject of trypanosome pathogenicity Wenyon[54] has an interesting comment; "Pathogenic trypanosomes give rise to disease in man or domestic animals and they do so not because these are their natural hosts, but because the man or animal is susceptible to inoculation with a trypanosome which in its nautral host is non-pathogenic."

Acting on the hypothesis that *T. lewisi* may not, after all, be specific to the rat, but merely a specialized strain of rodent trypanosome adapted to the medium provided by the rat's blood, Roudsky[45] attempted to render it virulent to other rodents. By successive subinoculation through a series of rat hosts he was successful in developing strains of *T. lewisi* inoculable to animals normally refractory to it such as the field mouse, guinea-pig, and rabbit. This method of animal passage is now standard bacteriological precedure for increasing the virulence of a microorganism relatively avirulent for a given animal. Although Roudsky

was able to do so, acquired virulence for one species does not necessarily mean that it is for others. With material obtained from an old blood-agar culture of T. lewisi he inoculated the first of a series of rats. As soon as the desired multiplication forms could be detected in the blood of the last injected rat subinoculation into the next susceptible animal followed until a series had been infected. Trypaosome blood obtained from the last member of the series was inoculable to the rodents mentioned above. With old culture tubes of T. lewisi which had been inoculated three months previously and incubated at 20°C to 25°C he infected a rat and started a new series from which he recovered a strain inoculable to a white mouse, the first member of a subsequent series of 32 mice some of which died of the infection, for the organism had become pathogenic; thereby illustrating the premise of Wenyon that microorganisms removed from their natural environment develop a pathogenicity when capable of surviving in the medium provided by a new host. The fact that the series was started from the culture tube, a medium in which the parasite behaves much as it does in the intermediate insect host, points to possible heightening of virulence as was discussed in the section on reproduction.

Roudsky[42] was successful in infecting mice with a strain of T. lewisi whose virulence was not increased by prolonged animal passage. After the inoculation of a rat with the rat trypanosome obtained from cultures he found modified forms.

After a second passage in blood-agar and reinoculation to rats persistent multiplication forms were found. It is quite possible that this strain developed a peculiar resistance to ablastin or may perhaps have been so modified that its antigens did not induce the production of ablastin by the host organism.

The animal usually utilized as host for T. lewisi in the laboratory is the tame rat. In his work Francis[18] used white and spotted rats and was able to infect guinea-pigs with normal T. lewisi. Procedure in securing blood from an animal found to be infected and injecting it to a laboratory animal is simple. Francis and Wenyon[54] suggest mixing one to three drops of blood with a small amount of sterile physiological saline which is injected intraperitoneasily. It is convenient to nick the ear or tail of an anesthetized wild rat with a sharp scalpel after having swabbed the area with an antiseptic, the blood being collected in a sterile vial or watchglass containing the saline solution. Laveran and Mesnil[28] recommend defibrination or citration of the blood before injection; the latter is probably the best practice since the blood would clot in the syringe unless an anticoagulant had been added. Only the speed gained with experience would be sufficient to withdraw and inject blood before clotting occurred.

Laveran and Mesnil[28] advise examining blood smears to ascertain the condition of the parasites since the success

of inoculation depends on the vitality of the microorganisms injected. Blood taken within two weeks after the establishment of infection will do but Laveran and Mesnil proably refer to the advanced stages of infection when degenerative forms with little virulence may be present. In bacteriological work an initial inoculum of minimum size is required to establish an infectious process but Laveran and Mesnil go so far as to say that trypanosomes in minute doses are sufficient, a single trypanosome being capable of bringing about infection. Whether the latter view is mere speculation or the result of careful experimentation is not stated by the authors but, in my opinion it is more acceptable to say that a minimal dose rather than a single organism is required.

Time required for appearance of trypanosomes in the peripheral circulation after inoculation depends on factors such as degree of infestation, developmental stage of the parasites, and host vitality. When he used heavily infected blood Francis[18] found the parasites in tail blood the second day after inoculation; MacNeal[31] found adult forms in three days. It is generally agreed that the peak of infection is reached the sixth or seventh day after inoculation, no more multiplication forms being apparent. It is advisable to subinoculate immediately before, during, or closely after the height of an infection is reached, for the parasites are then at a maximum state of virulence.

Intraperitoneal injection is universally favored for subinoculation it being the method requiring the minimum period of time for the appearance of trypanosomes in the general circulation. Until fairly recently the views of Laveran and Mesnil[28] regarding the early incubation of T. lewisi were accepted. They considered infections to be in three arbitrary stages when brought about intraperitoneally. The reproductive phase in the peritoneum in which many multiplication forms are present for three or four days in followed by the appearance of such forms in the blood until the end of the 8th day. The third stage is characterized by the presence of thin, non-dividing, adult forms until the termination of the infection. Superficially, this grouping of the phases of infection is valid, but Francis[18] takes exception to the theory that the peritoneum is the chief area for the initial incubation as upheld by Laveran and Mesnil and Rabinowitsch and Kempner.[41] He considers the blood to be the favored medium for multiplication. Small, immature forms pass to the blood stream via the lymphatics, while rosette forms are delayed therefrom by their size as well as other multiplying forms. In substantiation Augustine(1941) states[1] that T. lewisi injected intraperitoneally is absorbed by the lymphatics and passes readily through lymph nodes. Trypanosomes are apparent in the thoracic duct 2 hours after injection of infected blood. According to him there is no evidence of cellular reaction against the trypanosomes in

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nodes or channels. When division is complete then the circulatory system is reached where development is continued. Augustine presumably used adult forms and says nothing about the delayed entrance of rosettes into the lymphatics. The choice of either blood or peritoneum as the main site of development is relative, the factor preventing division forms from gaining access to the blood via the lymphatics being their size. This accounts for Laveran and Mesnil's statement that there are more multiplication forms in the peritoneal fluid than in the blood. When fission is complete the dividing forms of the peritoneal fluid are of the same identity as the small, immature trypanosomes seen in the blood. As in many of the controversial points cropping up in parasitology, the issues at stake are but extremities of the same thing, the whole matter being one of relativity so that the blood medium of Francis and the peritoneal fluid of Laveran and Mesnil must both be considered if one is to arrive at a complete picture of the initial stages of intraperitoneal infection.

To complement the foregoing discussion the views of MacNeal[31] are essential. He is convinced that early multiplication forms are harbored in the small capillaries of the internal organs so that the daughter cells have the advantage of higher temperature. To substantiate this view he found that a higher proportion of developmental forms are found in streak cultures of internal organs made the third

day after inoculation. In connection with migration to the capillaries of internal organs in early development, a similar phenomenon was observed by Rabinowitsch and Kempner[41] in white rats with old infections which suddenly exhibited a complete absence of trypanosomes in smears prepared from the peripheral blood. Such rats were autopsied and smears made from visceral blood were negative. However, kidney and liver emulsions injected intraperitoneally to young rats produced trypanosomiasis, the only plausible explanation being migration to the small, internal blood vessels where optimum conditions for survival prevail, the parasite remaining inactive and dormant once the height of infection has been attained and the infectious process is drawing to an end.

Recent work, however, has modified the older opinions regarding the early phases of intraperitoneal infection. Augustine[2] in 1941 stated that injected trypanosomes pass immediately to the blood stream no multiplication occurring until two days after inoculation and is therefore in partial agreement with Francis. According to the former, length of incubation period depends on two variable factors. The number of parasites injected and the developmental stage in which they happen to be. In his experiments Augustine used adult forms for injection and therefore, found no multiplication forms in the peritoneal cavity. This observation has led him to believe that Laveran and Mesnil[28] used parasites

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undergoing multiplication which would account for the presence of such forms in the peritoneal fluid. Reproduction, then, occurs primarily in the circulating blood.

Early development as advocated by MacNeal is wholly denied by Augustine. Although it is possible, however, that latent infections take refuge in the internal organs, X-irradiation[35] which definitely weakens resistance to T.lewisi has not produced evidence for existence of latent infection when administered to clinically recovered rats.

Beside the intraperitoneal there are other portals of entry through which artificial infections may be conveyed. Subcutaneous injection of infected blood is not as rapid as the abdominal path but Francis[18] states that a more severe infestation results. No reason is given. He also succeeded in infecting chloroformed rats by intrastomacheal injection of trypanosome blood with a well-oiled catheter. Average time for appearance of parasites in the blood was six days. Transmission by feeding the entire blood of an infected rat as well as the feeding of an infected wild rat to previously non-infected animals was successful. To repeat material discussed above in the section on life-history Rabinowitsch and Kempner[41] obtained negative results by the intrastomacheal injection method and noted that infection occurred when an infected tame rat was eaten by a non-infected wild rat after a heated battle. Francis interpreted the origin of the infection to be intrastomacheal, but the German

workers considered it to be wound contamination with infected blood. Therefore, intrastomacheal injection with infected blood should be further investigated before it is considered to be as effective as other experimental portals of entry.

It is generally agreed that T. lewisi is non-pathogenic, but Laveran and Mesmil[28] have observed splenomegaly and lymph gland enlargement in infected rats, conditions symptomatic of pathology. Duca (1939)[15] experimenting with young rats concluded that T. lewisi is pathogenic for rats 25 days old or younger and may cause death. A marked secondary anemia was observed in these young rats and is believed to be the major factor contributing to their death.

The remarks of Musgrave and Clegg[34] on the subject of pathogenicity deserve mention. Although they believe T. lewisi to be non-pathogenic as a result of the examination of thousands of rats in Manila they still are not fully positive. In their research it was found that a small percentage of rats harbored T. evansi. Autopsy of rats dying of a T. lewisi infection revealed splenomegaly, general enlargement of the lymphatics, especially in the inguinal region. In view of the fact that T. evansi had been found in some rats it was suspected of causing death, but animal experiments disproved this theory. Their comment is interesting:

> It is certain that rats in Manila may be infected both with a trypanosome harmless by inoculation to other animals and with one pathogenic for them...we are not fully

> convinced that T. lewisi is always harmless for rats or that some of the rats in Manila do not even harbor a third species of parasite.
> 42-45

Roudsky's work is significant as it points to the possibility of a common ancestral rodent trypanosome. The parent of the numerous species in existence today, the hypothetical primitive ancestral type became adapted to the special media provided by the various rodent hosts until a number of species was evolved. The same concept on a smaller scale was conceived by Rabinowitsch and Kempner[41] who considered the rat and hamster trypanosomes to be varieties of the same species. In a similar vein of thought Roudsky[43] described the implication of his work with the T. lewisi strain of increased virulence (T. lewisi renforcé). I believe it worth while to reproduce his comments here:

> Il résulte de ces expériences que le T. lewisi peut dans certaines conditions s'acclimater chez la souris; on doit, par suite, se demander si les trypanosomes non pathogènes des petits mammifères qui ont une si grande resemblance morphologique entre eux it avec T. lewisi n'appartiennet pas d'une meme espèce qui s'est si bien acclimaté chez ses differentes hôtes qu'elle a perdu la faculté de passer par inoculation directe d'une espèce animale à une autre.

The preceding speculations, although abstractions, may have immunological implications. The antibodies of the various rodents sufferring with trypanosomiasis may possibly be chemically allied because of their common antigen lineage. A dramatic illustration in support of such a hypothesis is

the immunization of white mice to T. duttoni infections by vaccination with killed T. lewisi.

It has been demonstrated that other factors are concerned with virulence and resistance in T. lewisi infections. Bilateral gonadectomy[38] reduces resistance while addition of copper and iron[39] to the diet or implantation in young rats of the pituitary[23] from adult non-immune rats heightens it. Injection of oestrin[23] to young rats substantially increases resistance. Endocrine factors in this case are apparently concerned with the natural increased resistance of adult rats, hormone equilibrium evidently being an important factor in immunity.

In Vitro Cultivation

A further aid to the study of trypanosomes in the laboratory is cultivation in the test tube. Novy and MacNeal[54] were the first to devise a blood-agar medium for the cultivation of trypanosomes. Nutrient agar was the type used by them, but the N.N.N. medium used today contains simple agar, a modification contributed by Nicolle. According to Wenyon[54] the "...development which occurs in the culture tube is an imitation of the invertebrate cycle of the trypanosome." By subculture the parasites can be maintained indefinitely and the phases from leishmania to crithidia can be seen. In cultures subinoculated every 30 days Delanoe[14] found trypaniform individuals in which

adult arrangement of kinetoplast and nucleus was present. Such forms were numerous in 6th or 7th generation subcultures and were frequently arranged in rosettes of 8 to 10 elements.

The blood of the rat, guinea-pig and rabbit have been found to be suitable for the preparation of N.N.N. medium. It may be obtained from the ear, a vein, or the heart, carotid artery or jugular vein may be utilized for puncture, the operation being performed under aseptic conditions to eliminate bacterial contamination.[54] There are four methods as outlined by Wenyon[54] for obtaining and adding blood. The immediate transfer of blood to the tube after direct, cardiac puncture is difficult since clotting may intervene. A vaseline-ether coating inside the syringe helps delay coagulation, or before being placed in culture tubes blood may be placed in a sterile bottle containing glass beads, defibrination being accomplished by shaking. Wenyon[54] says that animals can withstand repeated cardiac punctures under anesthesia. An animal can be bled aseptically to death under anesthesia, blood passing directly to a sterile jar containing glass beads for defibrination, the flow of blood being regulated by mild suction within the jar by means of an aspirator. A handy method eliminating the use of sterile syringe, tubing, and bottle is the incision of the marginal vein of a rabbit's ear, the blood being allowed to drop directly into the culture tube. So that the procedure be aseptic the ear is shaved over the marginal vein,

treated with antiseptic, and coated with hot paraffin. A Pressure clip is applied to the margin of the ear at its base and about twenty drops are mixed with a tube of liquid agar. When larger amounts of blood are used defibrination with sterile citrated physiological saline is probably easier than the glass bead method. Mixtures of citrated blood will keep the parasites alive several days, longevity being increased by refrigeration or addition of foreign sera according to Laveran and Mesnil.[28]

The composition of N.N.N. medium can be found in most texts dealing with bacteriological technique. When prepared, the mixture is poured to the height of 3 or 4 centimeters in the tube, plugged, and autoclaved. After sterilization and cooling, the tubes are filled with 2 to 3cc of blood while the agar is still liquid. With the arm held at the side the blood agar in the tube is mixed by a rotary motion and the tube is then placed in a sloped position to cool so that one side of the tube remains free of the medium. Wenyon suggests incubating the tubes at 37° C for 24 hours for a twofold purpose - the condensation liquid which will be inoculated is accumulated at the bottom of the tube and at the same time bacterial growth is encouraged to prove sterility. To prevent drying, the plugged mouths of the tubes may be dipped in hot paraffin or covered with a snug rubber cap.[54]

If there is to be a lapse of time between preparation

of medium and inoculation it is advisable to keep the tubes refrigerated for preservation. As an added preventive measure against contamination Mathis[54] suggests heating the blood agar to 75°C to 100°C for one half to one and a half hours one or more times, but I should think careful technique in preparation of the medium followed by incubation would be sufficient.

With an inoculating loop infected blood obtained from a convenient area pricked or incised aseptically is transferred to the condensation liquid at the bottom of the culture tube and incubated at 22 to 25°C. Growth occurs in the liquid and on the surface of the agar. If blood samples from internal organs are desired puncture with sterile needle and syringe may be done. MacNeal[31] advises incubation at 30°C and although Wenyon suggests subculture every 2 weeks he states that in 3 to 4 weeks the culture teems with all stages of the insect phase of the life-history, about a year being required for complete adaption to the medium. He describes the growth of glistening-white colonies on the blood-agar surface above the level of condensation liquid. Curiously enough, he has seen culture rosettes with flagella directed centrally.

Observation and slide Preparations

The final step in experimental work with trypanosomes after animal subinoculation and artificial culture is the study and observation of living organisms, dry and wet-fixed

films and tissue sections. Although the techniques required are standard knowledge it might be well for the sake of completeness to mention briefly some of the main points.

If a wild rodent host is used, the puncture or incision is usually made on the tail when the animal is safely anesthetized. Hanging-drop preparations made by mixing fresh blood with citrated, physiological saline are used to ascertain the presence of the parasite or for the observation of agglutination phenomena. With a magnification of about 250 the trypanosome can be recognized by the rotary motion of the erythrocytes. Francis[18] suggests allowing the blood to coagulate, whereupon the trypanosomes pass into the serum and are more easily observed. Another method of concentration is centrifuging of citrated blood, the parasites being found in the middle or leucocyte layer.[28]

When studying ordinary preparations on a plane slide there are several factors which aid in observation. Support of the cover slip by hairs to avoid pressure and sealing its edges with vaseline to prevent evaporation are helpful. For prolonged observation Wenyon suggests enclosing the microscope in a warm chamber at the temperature of the host. Reduction of temperature will decrease motility. Intravitam staining facilitates observation. Methylene blue and neutral red in dilute concentrations may be either mixed with the sample or allowed to dry on a slide before the sample is placed on the same area.[54] Wenyon notes that

at any time during observation the cover slip may be removed and a permanent smear prepared. Dark-field illumination is necessary for observation of the flagellum.[54]

The method used for preparation of smears for blood work is the same for observation of blood parasites. Before drying it is common practice to expose the smear to osmic acid vapor briefly for better fixation. If an aqueous stain is to be used immersion in absolute ethyl alcohol for about five minutes is advised before the smear is allowed to dry.[54] Giemsa and Wright's stains are the most common used in dry-film work. Ten drops of Giemsa stain to ten cubic centimeters of neutral distilled water is the recommended concentration. The staining fluid is transferred to a Petri dish; the slide is immersed in it face down supported by glass rods or match-sticks.[54] For observation of the flagellum Wenyon advises staining for one or more hours.

For wet-fixed preparations the slide is dropped into any of the common fixatives used for histological work before drying. After fixation the smears are passed through ascending concentrations of ethyl alcohol. If an aqueous stain is to be used the smear must be passed through a descending alcohol series as is done with tissue sections. Clearing in xylol after several changes of absolute alcohol is followed by mounting in balsam.[54] The techniques of fixation, sectioning, and staining employed in histological work are the same, according to Wenyon, used for preparation

of tissues containing trypanosomes. Wenyon stresses the importance of careful fixation and removal of the fixative in tissue section work.

VII. EXPERIMENTAL TRYPANOSOMIASIS IN RODENTS

Chemotherapy and Antibiosis

Several rodents have been used a number of years in experimentation with pathogenic trypanosomes of higher mammals including man. Besides serving for morphological studies, the artificial rodent hosts have been utilized in pharmaceutical research and have been essential to the development of the various specifics now in use against the trypanosomiases. The white rat, guinea-pig, rabbit, and white mouse are the rodents chiefly employed for the maintenance of laboratory infections; besides the great amount of chemotherapeutic work, experiments employing antibiotic phenomena have been performed on laboratory rodents.

Galliard[20] succeeded in effecting a complete cure in a mixed T. brucei and Treponema crocidurae infection in two white mice. The second day following inoculation with the mixture spirochaetes appeared in the peripheral blood. Several months later, however, subinoculation of blood to fresh animals produced negative results. A mutual antibiosis is believed to be responsible for the disappearance of both organisms. Vinzent[49] found that a spirochaete of the white mouse protects the guinea-pig, rabbit, young rat, and mouse against T. somaliense infections, a phenomenon which ceases upon the termination of the spirochaetosis. Although it would be irrelevant to discuss organic arsenical

drugs used in laboratory experiments with rodents it is appropriate to mention Ehrlich's ideas on the parasiticidal action of organic arsenicals.[19] He considered the possibility of affinity between groups in the drug and groups in the protoplasm of the organism with subsequent toxic action of other groups upon the parasite as well as the interference by the drug on enzyme action necessary to metabolism. Finally, Ehrlich thought that arsenicals might render the parasite more susceptible to host reactions. Recently Augustine[4] attempted therapy with penicillin sodium, but it was completely ineffective.

VIII. FINDINGS AND CONCLUSIONS

In the study of trypanosome-host relationship one is constantly reminded of the differences and resemblances between trypanosome and bacterial immunology. Generally speaking, protozoan infections do not confer immunity, but some do, including T. lewisi. Experimental work with formolized T. lewisi vaccines and immune sera have been successful as have bacterial vaccines and homologous immune sera in other animals including man. The first immune reaction to T. lewisi to be observed was agglutination, unlike the bacterial variety in that the agglutinated individuals usually retain their motility and sometimes escape from rosettes. As was pointed out in the section on immunity final destruction of T. lewisi is now believed to be by macrophage phagocytosis chiefly in the liver and spleen. Therefore one of the host responses elicited by T. lewisi is opsonin production. Excepting ablastin formation all the immune reactions including agglutination and macrophage stimulation are believed caused by a single trypanocidal antibody. Antibodies combatting bacterial infections usually induce the formation of a single antibody. An interesting serological phenomenon was pointed out in the case of T. duttoni and T. lewisi. Formolized suspensions of rat trypanosome vaccinia rendered white mice refractory to T. duttoni, a natural parasite of mice. Such group reactions have been

demonstrated in bacteriological experiments, while seemingly unrelated substances (alcoholic extract of beef heart and cholesterol, and T. pallidum) contain elements similar to specific bacterial or spirochaetal antigens. It is possible that organ extracts or special chemical substances may be discovered which will induce ablastin and trypanocide formation so that rats will be refractory to subsequent injection of live T. lewisi. Further investigation of group reactions in rodent trypanosomes should be worthwhile and may lend further support to the hypothesis that rodent trypanosomes are descended from a primitive, ancestral type. Experiments with trypanosomes of increased virulence similar to the work done by Roudsky may reproduce a strain generally inoculable to rodents.

Although Delanoe observed trypaniform individuals in his cultures, artificial growth of trypanosomes has not yet been successful in supporting adult forms - - they always revert to the phases of the insect cycle. Dr. G. Young has suggested that the development of a medium suitable for maintenance of adult forms would be a worthwhile research problem.

Investigation of the chemical and biological significance of insect passage bears looking into. The ending of arsenic-fastness by passage through the rat louse by Gonder is a clue to possible further phenomena occurring in the insect vector. The exact reason for the great morphological

changes which occur in the rat flea cycle is not known. Antibiotic reactions between trypanosomes and spirochaetes have been shown; antibiosis may be the key to the cure of spirochaetal and trypanosome diseases.

IX. ABSTRACT

The trypanosome was discovered in 1841 by Valentin of Berne in the blood of the trout. It was Lewis in India who in 1878 discovered trypanosomes in the blood of the rat and in doing so precipitated a new interest in mammalian trypanosomes. Cytological and morphological studies followed Lewis' work until Dr. Forde discovered a flagellated organism in the blood of a European supposedly suffering with malarial symptoms and Dr. Dutton recognized it to be a trypanosome. Not until the investigations of Castellani in 1903 was the relation of trypanosomes to human sleeping sickness realized, while Bruce demonstrated the role of the tsetse fly in the transmission of the disease.

After some years of indecision the trypanosome and closely related haemoflagellates were placed in the family, trypanosomidae on the basis of mutual morphological characteristics. Since trypanosomes experience several developmental metamorphoses varying from leishmania to true trypanosome phases it is thought that the evolutionary path of development is shown by the changes occurring in the insect vector. They correspond, more or less, with the changes experienced by the mammalian embryo in utero which are thought to indicate the line of mammalian evolution.

Adult trypanosomes are long, fusiform organisms with an anterior nucleus and posterior kinetoplast. The latter

IX. ABSTRACT

is a compound structure composed of a blepharoplast and parabasal body. Taking root in the blepharoplast the axoneme forms the lateral border of the undulating membrane and rejoins the main body at the anterior portion where it projects as the free flagellum. It is generally believed that trypanosomes because of the complexity of the invertebrate life-cycle are primarily insect parasites adapted to the vertebrate blood medium.

Essentially, multiplication of T. lewisi in the vertebrate host consists in the distribution of nuclear, kinetoplastic and protoplasmic material to the daughter individuals. Although the authors differ in interpretation there are basically two methods of multiplication. The first is simple longitudinal fission in which the blepharoplast is the first to divide, causing the parabasal body to follow suit. The undulating membrane cleaves and a new flagellum is developed for one of the daughter individuals. The second methods consists in multinucleation and formation of kinetoplasts corresponding to the number of nuclei. Flagella undulating membranes are formed while cleavage of the protoplasm follows from the periphery inwards.

Rabinowitsch and Kempner proved that C. fasciatus, the rat flea, is the vector of rat trypanosomiasis while Noller and Wenyon showed that transmission is effected by the rat ingesting infective flea faeces. Minchin and

Thompson worked out the detailed cycle of T. lewisi in the rat flea. Development therein is, essentially the formation of a crithidiomorphic individual in the stomach with transformation to a crithidial and subsequently trypaniform individual in the rectum. It is the latter form which is infective to the rat. Existence of sexual phenomena in the rat louse is claimed by Prowazek and Gonder discovered that passage through the rat flea brings arsenic-fastness to an end. Infection can only be attained by ingestion of trypaniform individuals present in faecal contents when the developmental cycle is complete.

When mixed with trypanosome blood the serum of rats recovered from infection will agglutinate the organisms. Agglutination is believed to be the first indication of immune reaction on the part of the host. It has long since been shown that two antibodies are involved in T. lewisi infections: ablastin, a reproduction-inhibitory substance, and a trypanocidal substance. Final destruction of the parasite is thought to be accomplished by liver and spleen macrophage phagocytosis. The protective properties of homologous immune serum and formolized trypanosome vaccine have been demonstrated.

In addition to the well-known rat parasite, trypanosomes have been found in a number of rodents all over the world. The wide distribution of these parasites and their morphological resemblances suggest a hypothetical rodent-trypanosome

ancestor which adapted itself to the special media of the various rodent species until a number of trypanosome species was evolved. Partial support to this hypothesis was given by Roudsky's work with trypanosomes of increased virulence. By rapid animal passage he developed a strain inoculable to mice, rabbits and guinea pigs.

Intraperitoneal injection is universally favored for inoculation experiments. Augustine has shown that trypanosomes injected intraperitoneally pass immediately *via* the lymphatics to the blood stream where multiplication takes place. It is generally agreed that *T. lewisi* is non-pathogenic though pathogenic strains have been encountered.

In vitro cultivation of T. lewisi in slant cultures of blood-agar may be achieved with N.N.N. medium. All the stages of the insect cycle are displayed in the test-tube. Strict aseptic technique in preparation of blood agar and inoculation is necessary. Subinoculation of cultures will maintain them indefinitely.

Trypanosomes may be examined *in vivo* or in wet and dry-fixed blood smears or tissue preparations. The standard histological methods are used in such work. The existence of antibiosis between trypanosomes and spirochaetes has been demonstrated.

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